

Table 3: Chromosomal Location of DNA Probes Utilized

<u>Probe</u>	<u>Locus Symbol</u>	<u>Location</u>	<u>Restriction Enzyme</u>	<u>Reference</u>
pJO71H-A	D5S20	5p13	EcoRI	1
p105-153A	D5S39	5q11.2-q13.3	MspI	1
$\pi$ 227	D5S37	5q21	PstI	1
C11p11	D5S71	5q14-q21	TaqI	1
M4	D5S6	5q11.2-q13.3	BamHI	1
J0205H-C	D5S22	5q34-qter	MspI	1
pYNZ22	D17S30	17p13.3	BamHI	2
pYNH37.3	D17S28	17p13.3	TaqI	1
pTHH59	D17S4	17q23-q25.3	TaqI	1
L2.7	D18S6	18p11	PstI	1
pHH64	TTR	18q11.2-q12.1	MspI	1
DCC1.9	DCC	18q21.3	EcoRI	4
p15-65	D18S8	18q21.3	MspI	3
pERT25	D18S11	18q23	PstI	1

1. Cytogenetics and Cell Genetics 58: 1-2200 (1991).
2. Nakamura Y: Nuc Acids Res 16:4707 (1987).
3. Marthens F, et al. Nuc. Acids Res 15:1348 (1987).
4. Vogelstein, unpublished results.

Table 4: Frequency of Allelic Loss on Chromosomes 5, 17 and 18

Chromosome	Loss		No Loss (No.)	NI (No.)
	No.	% *		
5p	9	20	36	46
5q	40	46	47	4
5	42	48	46	3
17p	59	69	26	6
17q	29	45	36	26
17	61	69	28	2
18p	24	69	11	56
18q	59	69	27	5
18	62	70	27	2

NI = non-informative

\* percentage refers to the number of tumors demonstrating loss of heterozygosity divided by the total number of tumors that were informative for the DNA probes utilized.

Table 5: Association of DNA Alterations with Loss of Heterozygosity

		NL	L		
		5q			
MFD 27	Neg	34 (33)	37 (36)	p=0.025	
	I	11 (8)	1		
	II	1	2		
		17p			
MFD 41	Neg	17 (16)	56 (55)	p<0.0005	
	I	7 (6)	1 (0)		
	II	2	0		
		18q			
MFD 26	Neg	15 (14)	53 (51)	p<0.0005	
	I	10 (8)	0		
	II	1	4		

NL = no loss

L = loss

( ) synchronous tumors omitted



Table 7. Clinicopathologic findings in MTS patients with tumors exhibiting microsatellite instability (Group I: cases 4, 6, 9, 23, 24, and 25) and with tumors not exhibiting microsatellite instability (Group II: cases 3, 5, 11, 17, 19, 21, and 22).

Case #	Number of sebaceous tumor(s)	Other skin tumors	Visceral malignancy			
			Site	Histology	Stage	Age°
4	5*	BCC KA* SK (12) VK (14)	Cecum*	ACA	B2	53
6	7*	AK (>50) IFK KA SCC (2) SK (3) VK	Ascending colon Rectosigmoid colon* Bladder Inner ear Kidney* Bladder Kidney Liver	? (outside institution) ACA TCC SCC RCC TCC RCC Metastatic ACA	? B2	42 47 49 72 73 74 74 74
9	5*	BCC (2) KA (2) SCC (4) SK VK (3)	Transverse colon Lymph node* Ascending colon* Lymph node	ACA CLL ACA CLL	B2 B2	38 62 67 69
23	6*	AK (2) SCC VK	Rectum* Ascending colon Transverse colon Kidney (renal pelvis)* Prostate	ACA ACA ACA TCC ACA	B1 B2 B2	42 64 65 70 73
24	5*	AK (2) EC (5) IFK	Cecum Rectum Liver Ovary Cecum* Rectum*	ACA ACA Metastatic ACA Adenoacanthoma ACA ACA	C2 C2 D B2 B1	23 29 30 33 51 64
25	1*	AK (3)	Descending colon Ascending colon* Retropertoneum	ACA ACA Metastatic ACA	C2 B1 D	40 52 62
3	1*	BCC (2)* Melanoma SK	Bladder	TCC		79
5	1	....	Colon (splenic flexure) Colon (hepatic flexure)* Peritoneum	ACA ACA Metastatic ACA	C2 C2 D	69 79 80
11	1	....	Cecum* Lymph node (groin)	ACA NHL	B2	79 93
17	1	EC SK	Stomach* Cervix Stomach*	NHL In situ SCC NHL		62 64 69
19	1*	EC	Breast*	ACA		68
21	1	AK (2)	Ascending colon* Jejunum* Peritoneum	ACA Metastatic ACA Metastatic ACA	A D D	68 68 68
22	1*	....	Breast*	Invasive ACA		74

## KEY TO ABBREVIATIONS AND SYMBOLS

ACA-adenocarcinoma  
AK-actinic keratosis  
BCC-basal cell carcinoma  
CLL-chronic lymphocytic leukemia  
EC-epidermoid cyst  
IFK-inverted follicular keratosis  
KA-keratoacanthoma  
NHL-non-Hodgkin's lymphoma

RCC-renal cell carcinoma  
SCC-squamous cell carcinoma  
SK-seborrheic keratosis  
TCC-transitional cell carcinoma  
VK-verruca keratosis  
\*PCR data obtained  
†Stage of colorectal cancer  
°Age at diagnosis of visceral malignancy

Table 8. Results of assays for microsatellite instability in the various tumors.

## PATIENTS WITH MICROSATELLITE INSTABILITY †

Case #	Tumor site (histology)*	# of loci demonstrating microsatellite instability
4	Cecum (ACA)	4/4
	Skin (SE)	4/4
	Skin (KA)	4/4
6	Rectosigmoid colon (ACA)	3/4
	Kidney (RCC)	0/4
	Skin (SA)	4/4
	Skin (SA)	3/4
9	Skin (SA)	4/4
	Skin (SA)	3/4
	Ascending Colon (ACA)	4/4
	Skin (SC)	4/4
	Skin (SC)	4/4
	Lymph node (CLL)	0/4
23	Rectum (ACA)	4/4
	Skin (SA)	4/4
	Skin (SA)	4/4
	Renal pelvis (TCC)	3/4
	Skin (SC)	4/4
	Prostate (ACA)	3/4
24	Cecum (ACA)	3/4
	Skin (SA)	4/4
	Rectum (ACA)	3/4
25	Ascending colon (ACA)	4/4
	Skin (SA)	3/4

## PATIENTS WITHOUT MICROSATELLITE INSTABILITY

Case #	Tumor site (histology)	# of loci demonstrating microsatellite instability
3	Skin (BCC)	0/4
	Skin (SC)	0/4
5	Hepatic flexure (ACA)	0/4
11	Cecum (ACA)	0/4
17	Stomach (NHL)	0/4
	Stomach (NHL)	0/4
19	Eyelid (Meibomian gland adenoma)	0/4
	Breast (ACA)	0/4
21	Ascending colon (ACA)	0/4
	Jejunum (metastatic ACA)	1/4
22	Eyelid (Meibomian gland carcinoma)	0/4
	Breast (ACA)	0/4

## KEY TO ABBREVIATIONS AND SYMBOLS

\*Tumors are ordered according to sequence of occurrence in patient.

†Instability at 3 of 4 loci

SA-sebaceous adenoma

SE-sebaceous epithelioma

SC-sebaceous carcinoma

See Table 1 for abbreviations of remaining tumor types.

Table 9. Summary of clinical features of patients with or without microsatellite instability.

## PATIENTS WITH MICROSATELLITE INSTABILITY†

Case #	Age of onset of first visceral malignancy (tumor type)	Survival following onset of first visceral malignancy (y)	Family history of cancer	Comments
4	53 (Cecum)	24	Yes	Alive, no evidence of malignancy at age 78.
6	42 (Ascending colon)	32	Yes	Death from renal cell carcinoma* at age 74.
9	38 (Transverse colon)	32	Yes	Death from CLL* at age 69.
23	42 (Rectum)	35	Yes	Death from Alzheimer's disease at age 76. No malignancy at death.
24	23 (Cecum)	37	Yes	Alive, no evidence of malignancy at age 70.
25	40 (Descending colon)	23	Yes	Death from suicide at age 63. Metastatic colon cancer at death.

## PATIENTS WITHOUT MICROSATELLITE INSTABILITY

Case #	Age of onset of first visceral malignancy (tumor type)	Survival following onset of first visceral malignancy (y)	Family history of cancer	Comments
3	79 (Bladder)	9	Yes	Death from metastatic melanoma at age 89.
5	69 (Splenic flexure)	11	No	Death from metastatic colon cancer at age 81.
11	70 (Cecum)	25	Yes	Lost to follow up at age 95. High stage lymphoma at that time.
17	62 (Lymphoma)	23	No	Alive, no evidence of malignancy at age 85.
19	68 (Breast)	11	No	Death at age 79 from "heart disease".
21	68 (Ascending colon)	1	Yes	Death from metastatic colon cancer at age 69.
22	74 (Breast)	1	Yes	Death from metastatic Meibomian gland carcinoma at age 75.

†Instability in at least 3 of the 4 loci

\* Only tumor not exhibiting microsatellite instability in this patient.

SEQUENCE LISTING

## (1) GENERAL INFORMATION:

- 5 (i) APPLICANT: Stephen N. Thibodeau  
Gary D. Bren
- (ii) TITLE OF INVENTION: TUMOR-SPECIFIC GENOMIC INSTABILITY  
10 AS A PROGNOSTIC INDICATOR
- (iii) NUMBER OF SEQUENCES: 18
- (iv) CORRESPONDENCE ADDRESS:
- 15 (A) ADDRESSEE: Patterson & Keough, P.A.  
(B) STREET: 1200 Rand Tower  
527 Marquette Avenue South
- 20 (C) CITY: Minneapolis  
(D) STATE: Minnesota  
(E) COUNTRY: USA
- 25 (F) ZIP: 55402
- (v) COMPUTER READABLE FORM:
- 30 (A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: Apple Macintosh  
(C) OPERATING SYSTEM: Apple Macintosh System 7.0.1
- 35 (D) SOFTWARE: WordPerfect 2.1.4 for the Macintosh
- (vi) CURRENT APPLICATION DATA:
- 40 (A) APPLICATION NUMBER:  
(B) FILING DATE:  
(C) CLASSIFICATION:
- 45 (viii) ATTORNEY/AGENT INFORMATION:
- (A) NAME: Mark S. Ellinger, Esq.
- 50 (B) REGISTRATION NUMBER: 34,812  
(C) REFERENCE/DOCKET NUMBER: 1144.01-WO-01



(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: 612/349-5743

5

(B) TELEFAX: 612/349-9266

41

## (2) INFORMATION FOR SEQ ID NO:1

## (i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 21  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
10 (D) TOPOLOGY: linear

## (viii) POSITION IN GENOME:

- 15 (A) CHROMOSOME/SEGMENT: 5q11.2-q13.3  
(B) MAP POSITION: D5S107

20 (xi) A SEQUENCE DESCRIPTION: SEQ ID NO:1:

GATCCACTTT AACCCAAATA C 21

25

## (2) INFORMATION FOR SEQ ID NO:2

## (i) SEQUENCE CHARACTERISTICS:

- 30 (A) LENGTH: 20  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
35 (D) TOPOLOGY: linear

## (viii) POSITION IN GENOME:

- 40 (A) CHROMOSOME/SEGMENT: 5q11.2-q13.3  
(B) MAP POSITION: D5S107

45

## (xi) A SEQUENCE DESCRIPTION: SEQ ID NO:2:

GGCATCAACT TGAACAGCAT 20

42

## (2) INFORMATION FOR SEQ ID NO:3

## (i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 20  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
10 (D) TOPOLOGY: linear

## (viii) POSITION IN GENOME:

- 15 (A) CHROMOSOME/SEGMENT: 17p12-p11.1  
(B) MAP POSITION: D17S261

## 20 (xi) A SEQUENCE DESCRIPTION: SEQ ID NO:3:

CAGGTTCTGT CATAGGACTA 20

25

## (2) INFORMATION FOR SEQ ID NO:4

## (i) SEQUENCE CHARACTERISTICS:

- 30 (A) LENGTH: 20  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
35 (D) TOPOLOGY: linear

## 40 (viii) POSITION IN GENOME:

- (A) CHROMOSOME/SEGMENT: 17p12-p11.1  
(B) MAP POSITION: D17S261

45

## (xi) A SEQUENCE DESCRIPTION: SEQ ID NO:4:

TTCTGGAAAC CTACTCCTGA 20

43

## (2) INFORMATION FOR SEQ ID NO:5

## (i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 20  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
10 (D) TOPOLOGY: linear

## (viii) POSITION IN GENOME:

- 15 (A) CHROMOSOME/SEGMENT: 18q  
(B) MAP POSITION: D18S34

## 20 (xi) A SEQUENCE DESCRIPTION: SEQ ID NO:5:

CAGAAAATTC TCTCTGGCTA 20

25

## (2) INFORMATION FOR SEQ ID NO:6

## (i) SEQUENCE CHARACTERISTICS:

- 30 (A) LENGTH: 20  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
35 (D) TOPOLOGY: linear

## 40 (viii) POSITION IN GENOME:

- (A) CHROMOSOME/SEGMENT: 18q  
(B) MAP POSITION: D18S34

45

## (xi) A SEQUENCE DESCRIPTION: SEQ ID NO:6:

CTCATGTTCC TGGCAAGAAT 20

44

## (2) INFORMATION FOR SEQ ID NO:7

## (i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 20  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
10 (D) TOPOLOGY: linear

## (viii) POSITION IN GENOME:

- 15 (A) CHROMOSOME/SEGMENT: 15q11-qter

## (xi) A SEQUENCE DESCRIPTION: SEQ ID NO:7:

20 TTGACCTGAA TGCACGTGCA 20

## 25 (2) INFORMATION FOR SEQ ID NO:8

## (i) SEQUENCE CHARACTERISTICS:

- 30 (A) LENGTH: 20  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
35 (D) TOPOLOGY: linear

## (viii) POSITION IN GENOME:

- 40 (A) CHROMOSOME/SEGMENT: 15q11-qter

## (xi) A SEQUENCE DESCRIPTION: SEQ ID NO:8:

45 TTCCATACCT GGCAACGAGT 20

45

## (2) INFORMATION FOR SEQ ID NO:9

## (i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 20  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
10 (D) TOPOLOGY: linear

## (x) PUBLICATION INFORMATION:

- 15 (A) AUTHORS: Peterson, M.G.  
Tanese, N.  
Pugh, B.F.  
Tjian, R.  
20 (B) TITLE: Functional domains and upstream  
activation properties of cloned human TATA  
binding protein  
(C) JOURNAL: Science  
25 (D) VOLUME: 248  
(F) PAGES: 1625-1630  
30 (G) DATE: 1990  
(K) RELEVANT RESIDUES: 352 - 371

## 35 (xi) A SEQUENCE DESCRIPTION: SEQ ID NO:9:

ACTGACCCCA CAGCCTATTC 20

40

## (2) INFORMATION FOR SEQ ID NO:10

## (i) SEQUENCE CHARACTERISTICS:

- 45 (A) LENGTH: 20  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
50 (D) TOPOLOGY: linear

46

## (x) PUBLICATION INFORMATION:

- 5 (A) AUTHORS: Peterson, M.G.  
Tanese, N.  
Pugh, B.F.  
Tjian, R.
- 10 (B) TITLE: Functional domains and upstream  
activation properties of cloned human TATA  
binding protein
- (C) JOURNAL: Science
- 15 (D) VOLUME: 248
- (F) PAGES: 1625-1630
- (G) DATE: 1990
- 20 (K) RELEVANT RESIDUES: 618 - 637

## (xi) A SEQUENCE DESCRIPTION: SEQ ID NO:10:

25 CAAGGGTGCA GTTGTGAGAG 20

30 (2) INFORMATION FOR SEQ ID NO:11

## (i) SEQUENCE CHARACTERISTICS:

- 35 (A) LENGTH: 21
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- 40 (D) TOPOLOGY: linear

## (viii) POSITION IN GENOME:

- 45 (A) CHROMOSOME/SEGMENT: 5q

## (x) PUBLICATION INFORMATION:

- 50 (A) AUTHORS: Spiro, L. et al.
- (B) TITLE: A CA repeat 30-70 Kb downstream from the  
adenomatous polyposis coli (APC) gene
- (C) JOURNAL: Nucleic Acids Res.
- 55 (D) VOLUME: 19

47

(F) PAGES: 6348 et. seq.

(G) DATE: 1991

5

(xi) A SEQUENCE DESCRIPTION: SEQ ID NO:11:

ACTCACTCTA GTGATAAATC G 21



48

## (2) INFORMATION FOR SEQ ID NO:12

## (i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 25  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
10 (D) TOPOLOGY: linear

## (viii) POSITION IN GENOME:

- 15 (A) CHROMOSOME/SEGMENT: 5q

## (x) PUBLICATION INFORMATION:

- 20 (A) AUTHORS: Spiro, L. et al.  
(B) TITLE: A CA repeat 30-70 Kb downstream from the  
adenomatous polyposis coli (APC) gene  
25 (C) JOURNAL: Nucleic Acids Res.  
(D) VOLUME: 19  
(F) PAGES: 6348 et. seq.  
30 (G) DATE: 1991

## (xi) A SEQUENCE DESCRIPTION: SEQ ID NO:12:

35 AGCAGATAAG ACAGTATTAC TAGTT 25

## 40 (2) INFORMATION FOR SEQ ID NO:13

## (i) SEQUENCE CHARACTERISTICS:

- 45 (A) LENGTH: 20  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
50 (D) TOPOLOGY: linear

## (viii) POSITION IN GENOME:

- 55 (A) CHROMOSOME/SEGMENT: 15q

## (x) PUBLICATION INFORMATION:

- 5 (A) AUTHORS: Thibodeau, S.N. et al.
- (B) TITLE: Microsatellite instability in cancer of  
the proximal colon
- 10 (C) JOURNAL: Science
- (D) VOLUME: 260
- (F) PAGES: 816-819
- 15 (G) DATE: 1993

## (xi) A SEQUENCE DESCRIPTION: SEQ ID NO:13:

20 TTGACCTGAA TGCACGTGTA 20

50

## (2) INFORMATION FOR SEQ ID NO:14

## (i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 20  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
10 (D) TOPOLOGY: linear

## (viii) POSITION IN GENOME:

- 15 (A) CHROMOSOME/SEGMENT: 15q

## (x) PUBLICATION INFORMATION:

- 20 (A) AUTHORS: Thibodeau, S.N. et al.  
(B) TITLE: Microsatellite instability in cancer of  
the proximal colon  
25 (C) JOURNAL: Science  
(D) VOLUME: 260  
(F) PAGES: 816-819  
30 (G) DATE: 1993

## (xi) A SEQUENCE DESCRIPTION: SEQ ID NO:14:

35 TTCCATACCT GGGAACGAGT 20

## 40 (2) INFORMATION FOR SEQ ID NO:15

## (i) SEQUENCE CHARACTERISTICS:

- 45 (A) LENGTH: 24  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
50 (D) TOPOLOGY: linear

## (viii) POSITION IN GENOME:

- 55 (A) CHROMOSOME/SEGMENT: 17p

51

## (x) PUBLICATION INFORMATION:

- 5 (A) AUTHORS: Jones, M.H., and Nakamura, Y.
- (B) TITLE: Detection of loss of heterozygosity at  
the human TP53 locus using a dinucleotide  
repeat polymorphism
- 10 (C) JOURNAL: Genes Chrom. Cancer
- (D) VOLUME: 5
- (F) PAGES: 89-90
- 15 (G) DATE: 1992

## (xi) A SEQUENCE DESCRIPTION: SEQ ID NO:15:

AGGGATACTA TTCAGCCCCGA GGTG 24